Qubit™ Protein Assay Kits

Catalog Numbers Q33211, Q33212

Pub. No. MAN0002349 Rev. B.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Qubit™ Protein Assay Kits make protein quantitation easy and accurate. The kits include concentrated assay reagent, dilution buffer, and prediluted BSA standards. Simply dilute the reagent using the buffer provided, add your sample (any volume from 1–20 µL is acceptable), then read the concentration using the Qubit™ Fluorometer. The assay is accurate for initial sample concentrations from 12.5 µg/mL to 5 mg/mL and exhibits low protein-to-protein variation (Figure 1). The assay is performed at room temperature, and the signal is stable for 3 hours. Common contaminants such as reducing reagents (DTT, β-mercaptoethanol), salts, free nucleotides, amino acids, solvents, or DNA (but not detergents) are well tolerated in the assay; some very slight modifications for the procedure are required for other contaminants (see “Contaminants tolerated by the Qubit™ protein assay” on page 4). In addition to the Qubit™ Protein Assay Kits described here, we also offer other kits for assaying dsDNA and RNA (see “Related products” on page 7).

Note: This Qubit™ assay kit can be used with any Qubit™ Fluorometer.

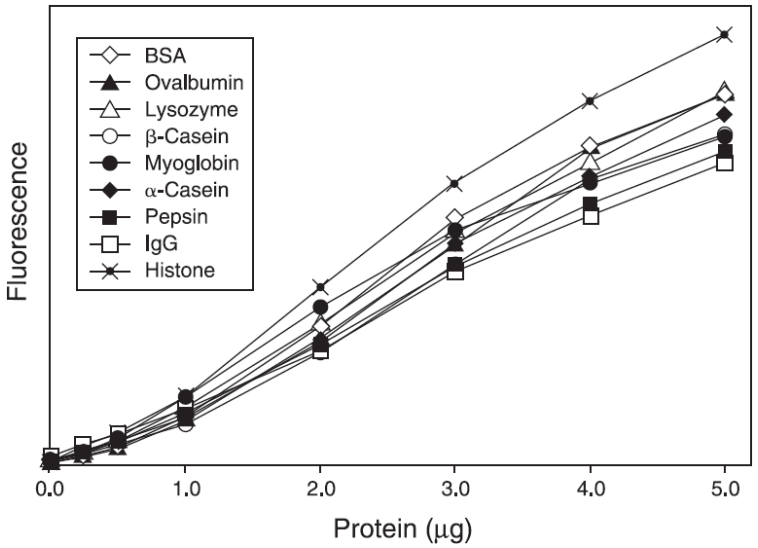

Figure 1 Low protein-to-protein variation in the Qubit™ protein assay.
Solutions of the following proteins were prepared, diluted, and assayed in the Qubit™ protein assay: bovine serum albumin (BSA), chicken-egg ovalbumin, chicken-egg lysozyme, bovine-milk β-casein, equine myoglobin, bovine-milk α-casein, porcine pepsin, mouse immunoglobulin (IgG), and calf thymus histone. Fluorescence was measured at 485/590 nm and plotted versus the mass of the protein sample. At 3 µg, the fluorescence variation was 12.4%, or 8.7% excluding the basic histone protein. Background fluorescence has not been subtracted.

Contents and storage

For use with the Qubit™ Fluorometer (all models)

Component	Cat. No. Q33211 (100 assays)	Cat. No. Q33212 (500 assays)	Concentration	Storage[1]
Qubit™ Protein Reagent (Component A)	300 µL	1.5 mL	200X concentrate in 1,2-propanediol	Room temperature Desiccate Protect from light
Qubit™ Protein Buffer (Component B)	60 mL	300 mL	Not applicable	Room temperature
Qubit™ Protein Standard #1 (Component C)	1 mL	5 mL	0 ng/µL in TE buffer with 2 mM sodium azide	≤4°C
Qubit™ Protein Standard #2 (Component D)	1 mL	5 mL	200 ng/µL in TE buffer with 2 mM sodium azide	
Qubit™ Protein Standard #3 (Component E)	1 mL	5 mL	400 ng/µL in TE buffer with 2 mM sodium azide	

[1] When stored as directed, kits are stable for 6 months.

Materials required not supplied

- Plastic container (disposable) for mixing the Qubit™ working solution (step 3 on page 5)
- Qubit™ Assay Tubes (500 tubes, Cat. No. Q32856) or Qubit™ Flex Assay Tube Strips (Cat. No. Q33252)

Guidelines for handling the Qubit™ Protein Reagent

No data are currently available that address the mutagenicity or toxicity of the Qubit™ Protein Reagent (Component A). This reagent is an organic dye and is provided as a solution in 1,2-propanediol. Treat the Qubit™ Protein Reagent with the same safety precautions as other materials with similar properties and dispose of the dye in accordance with local regulations.

Critical assay parameters

Assay temperature

The Qubit™ protein assay delivers optimal performance when all solutions are at room temperature. The Qubit™ assays are designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay (Figure 2). To minimize temperature fluctuations, store the Qubit™ protein reagent and buffer at room temperature and insert all assay tubes into the Qubit™ Fluorometer only for as much time as it takes for the instrument to measure the fluorescence; the Qubit™ Fluorometer can raise the temperature of the assay solution significantly, even over a period of a few minutes. Do not hold the assay tubes in your hand before reading because this warms the solution and results in a low reading.

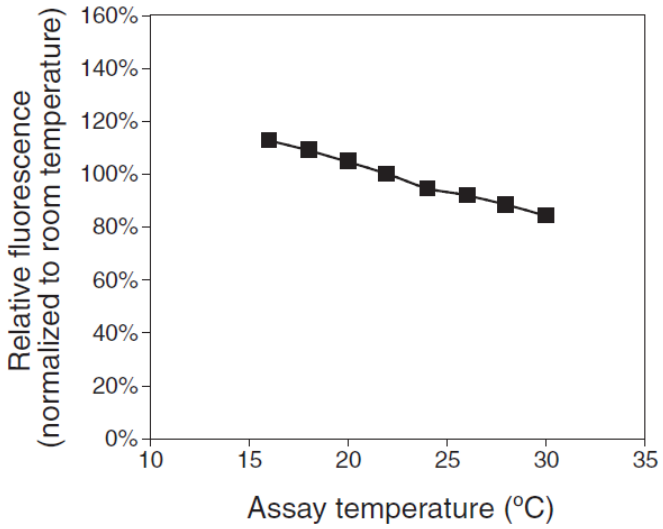

Figure 2 Plot of fluorescence vs. temperature for the Qubit™ protein assay.
The Qubit™ assays are designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay.

Incubation time

To allow the Qubit™ protein assay to reach optimal fluorescence, incubate the tubes for 15 minutes after mixing the sample or standard with the working solution. After this incubation period, the fluorescence signal is stable for 3 hours at room temperature. For the greatest accuracy of the Qubit™ protein assay, the incubation time of the samples should be within 10 minutes of the incubation time of the standards.

Photostability of the Qubit™ reagents

The Qubit™ reagents exhibit high photostability in the Qubit™ Fluorometer, showing <0.3% drop in fluorescence after 9 readings and <2.5% drop in fluorescence after 40 readings. However, if the assay tube remains in the Qubit™ Fluorometer for multiple readings, a temporary reduction in fluorescence will be observed as the solution increases in temperature (Figure 2). Note that the temperature inside the Qubit™ Fluorometer may be as much as 3°C above room temperature after 1 hour. For this reason, if you want to perform multiple readings of a single tube, remove the tube from the instrument and let it equilibrate to room temperature for 30 seconds before taking another reading.

Calibrate the Qubit™ Fluorometer

For each assay, you have the choice to run a new calibration or to use the values from the previous calibration. When you first use the instrument, perform a new calibration each time. As you become familiar with the assays, the instrument, your pipetting accuracy, and significant temperature fluctuations within your laboratory, you can decide the level of comfort you have using the calibration data stored from the last time the instrument was calibrated. Remember that the fluorescence signal in the tubes containing standards and samples is stable for no longer than 3 hours. See Figure 3 for an example of the calibration curve used to generate the quantification results.

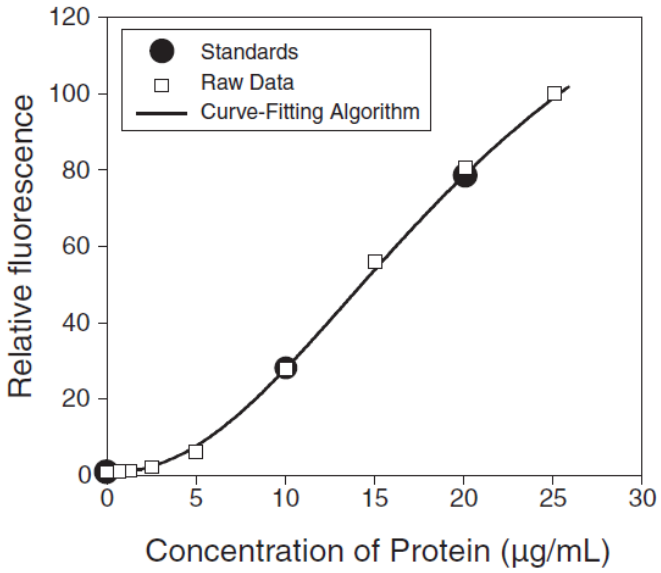

Figure 3 The curve-fitting algorithm used to determine concentration in the Qubit™ protein assay.
The Qubit™ Fluorometer generates concentration data based on the relationship between the three standards used in the calibration. This plot shows the line corresponding to the curve-fitting algorithm (a modified Hill plot) used in the calculation of concentration data for the Qubit™ protein assay. For reference, the positions of the standards and a set of data points from an actual experiment are shown superimposed onto the line, demonstrating that the curve-fitting algorithm gives accurate values for quantitation.

Contaminants tolerated by the Qubit™ protein assay

Table 1 Effect of contaminants in the Qubit™ protein assay, tested over a range of 1.25–25 µg/mL*

Contaminant	Final concentration in the assay	Concentration in 20-µL sample	Concentration in 10-µL sample	Result
Sodium chloride	20 mM	200 mM	400 mM	OK [1]
Magnesium chloride	2 mM	20 mM	40 mM	OK
Potassium chloride [2]	200 mM	200 mM	400 mM	OK
Calcium chloride[2]	2 mM	20 mM	40 mM	OK
Ammonium sulfate	5 mM	50 mM	100 mM	OK[1]
DTT	1 mM	10 mM	20 mM	OK[1]
β-Mercaptoethanol	1 mM	10 mM	20 mM	OK
EDTA	1 mM	10 mM	20 mM	OK
Sodium azide	1 mM	10 mM	20 mM	OK
HEPES, pH 7.4	5 mM	50 mM	100 mM	OK
Potassium phosphate, pH 7.4	5 mM	50 mM	100 mM	OK
PBS, pH 7.4	1 mM KPO_4 15 mM NaCl	10 mM KPO_4 150 mM NaCl	20 mM KPO_4 300 mM NaCl	Protocol modification required [3]
Sucrose	50 mM	500 mM	1 M	OK
Sucrose	100 mM	1 M	2 M	NR
Glycerol	1%	10%	20%	OK[1]
Imidazole	1.25 mM	12.5 mM	25 mM	OK
SDS	0.01%	0.1%	0.2%	OK[1]
SDS	0.02%	0.2%	0.4%	NR
Tween™ 20	0.001%	0.01%	0.02%	NR
Triton™ X-100	0.001%	0.01%	0.02%	NR
Amino acids [4]	100 µg/mL	1 mg/mL	2 mg/mL	OK
dNTPs[5]	100 µM	1 mM	2 mM	OK[1]
DNA	5 µg/mL	50 µg/mL	100 µg/mL	OK[1]
DNA	10% [6]	10%[6]	10%[6]	OK
DNA	50%[6]	50%[6]	50%[6]	NR

*BSA standards were assayed in the presence or absence of contaminants at the indicated final concentrations. Equivalent concentrations (approximate) in 20-µL or 10-µL sample volumes are also listed. Results are given as OK, usually less than 10% perturbation, or as NR (not recommended).

[1] An acceptable result, but with some distortion of the standard curve. For best results, add the same amount of contaminant to the standard samples.
[2] A precipitate was observed.
[3] For accurate results, add the same amount of PBS to the standard samples.
[4] A mixture of 19 amino acids.
[5] A mixture of dATP, dCTP, dGTP, and dTTP.
[6] For each data point, the DNA mass was a fixed percentage of the protein mass.

Prepare samples and standards

This protocol assumes that you are preparing standards for calibrating the Qubit™ Fluorometer. If you plan to use the last calibration performed on the instrument (see "Calibrate the Qubit™ Fluorometer" on page 3), you need fewer tubes (step 1) and less working solution (step 3).

1. Set up the required number of 0.5-mL tubes for standards and samples. The Qubit™ protein assay requires 3 standards.

   Note: Use only thin-wall, clear, 0.5-mL PCR tubes (Cat. No. Q32856) for the Qubit™ 4 Fluorometer and 8 × 200-µL tube strips (Cat. No. Q33252) for the Qubit™ Flex Fluorometer.

2. Label the tube lids.

   Note: Do not label the side of the tube as this could interfere with the sample read. Label the lid of each standard tube correctly. Calibration of the Qubit™ Fluorometer requires the standards to be inserted into the instrument in the right order.

3. Prepare the Qubit™ working solution by diluting the Qubit™ Protein Reagent 1:200 in Qubit™ Protein Buffer. Use a clean plastic tube each time you prepare Qubit™ working solution.

   IMPORTANT! Do not mix the working solution in a glass container.

   Note: The final volume in each tube must be 200 µL. Each standard tube requires 190 µL of Qubit™ working solution, and each sample tube requires anywhere from 180–199 µL. Prepare sufficient Qubit™ working solution to accommodate all standards and samples.

   For example, for 7 samples, prepare enough working solution for the samples and 3 standards: ~200 µL per tube in 10 tubes yields 2 mL of working solution (10 µL of Qubit™ reagent plus 1990 µL of Qubit™ buffer).

4. Add 190 µL of Qubit™ working solution to each of the tubes used for standards.

5. Add 10 µL of each Qubit™ standard to the appropriate tube, then mix by vortexing 2–3 seconds. Be careful not to create bubbles.

   Note: Careful pipetting is critical to ensure that exactly 10 µL of each Qubit™ standard is added to 190 µL of Qubit™ working solution.

6. Add Qubit™ working solution to individual assay tubes so that the final volume in each tube after adding sample is 200 µL.

   Note: Your sample can be anywhere from 1–20 µL. Add a corresponding volume of Qubit™ working solution to each assay tube: anywhere from 180–199 µL.

7. Add each sample to the assay tubes containing the correct volume of Qubit™ working solution, then mix by vortexing 2–3 seconds. The final volume in each tube should be 200 µL.

8. Allow all tubes to incubate at room temperature for 15 minutes, then proceed to read standards and samples (next section).

Read standards and samples

Follow the procedure appropriate for your instrument.

Read samples and standards with the Qubit™ 4 Fluorometer

For a more complete overview on using the Qubit™ 4 Fluorometer, please refer to *Qubit™ 4 Fluorometer User Guide* (Pub. No. MAN0017209), available for download at **thermofisher.com/qubit**.

1. On the home screen of the Qubit™ 4 Fluorometer, press **Protein** then select **Protein**. Press **Read standards** to proceed.

   Note: If you have already performed a calibration for the selected assay, the instrument prompts you to choose between reading new standards and running samples using the previous calibration. If you want to use the previous calibration, skip to step 5 below.

2. Insert the tube containing Standard 1 into the sample chamber, close the lid, then press **Read standard**. When the reading is complete (~3 seconds), remove Standard 1.

3. Insert the tube containing Standard 2 into the sample chamber, close the lid, then press **Read standard**. When the reading is complete, remove Standard 2.

4. Insert the tube containing Standard 3 into the sample chamber, close the lid, then press **Read standard**. When the reading is complete, remove Standard 3.

5. Press **Run samples**.

6. On the assay screen, select sample volume and units. Press the + or – buttons on the wheel to select sample volume added to the assay tube (1 µL–20 µL).

7. From the drop-down menu, select the units (µg/mL, mg/mL) for the output sample concentration.

8. Insert sample, close lid, then press **Read Tube**. When the reading is complete (~3 seconds), remove the sample tube. The instrument displays the results on the assay screen. The value displayed is the concentration of the sample.

9. Repeat step 7 until all samples have been read.

Read samples and standards with the Qubit™ Flex Fluorometer

For a more complete overview on using the Qubit™ Flex Fluorometer, please refer to *Qubit™ Flex Fluorometer User Guide* (Pub. No. MAN0018186), available for download at **thermofisher.com/qubit**.

1. On the home screen of the Qubit™ Flex, select **Protein**. Press **Read standards and run samples** to proceed.

   Note: If you have already performed a calibration for the selected assay, the instrument prompts you to choose between reading new standards and running samples using the previous calibration. If you want to use the previous calibration, skip to step 5 below.

2. Insert the tubes containing Standard 1 into the sample chamber, close the lid, then press **Read standard**. When the reading is complete (~3 seconds), remove Standard 1.

3. Insert the tubes containing Standard 2 into the sample chamber, close the lid, then press **Read standard**. When the reading is complete, remove Standard 2.

4. Insert the tubes containing Standard 3 into the sample chamber, close the lid, then press **Read standard**. When the reading is complete, remove Standard 3.

5. Press **Run samples**.

6. On the assay screen, select **Output sample units** from the drop-down menu. Press to deselect tube positions that do not contain a sample. Select **Next.**

7. Press the + or – buttons on the wheel to select sample volume added to the assay tube (1 µL–20 µL).

8. Insert sample, close lid, then press **Read Samples**. When the reading is complete (~3 seconds), remove the sample tubes. The instrument displays the results on the assay screen. The value displayed is the concentration of the sample.

9. For additional samples select **Add samples.**

10. Insert tubes and deselect tube positions that do not contain a sample. Select **Run samples.**

11. Repeat step 9 and step 10 until all samples have been read.

Related products

Table 2 Assays

Product	Quantitation range	Quantity	Cat. No.
Qubit™ Protein BR Assay Kit[1]	0.1–20 mg	100 reactions 500 reactions	A50668 A50669
Qubit™ Protein Assay Kit	12.5–5,000 µg	100 reactions 500 reactions	Q33211 Q33212
Qubit™ 1X dsDNA HS Assay Kit	0.1–120 ng	100 reactions 500 reactions	Q33230 Q33231
Qubit™ 1X dsDNA BR Assay Kit	4–4,000 ng	100 reactions 500 reactions	Q33265 Q33266
Qubit™ dsDNA HS Assay Kit	0.1–120 ng	100 reactions 500 reactions	Q32851 Q32854
Qubit™ dsDNA BR Assay Kit	4–2,000 ng	100 reactions 500 reactions	Q32850 Q32853
Qubit™ ssDNA Assay Kit	0.2–240 ng	100 reactions	Q10212
Qubit™ RNA IQ Assay Kit	N/A	75 reactions 275 reactions	Q33221 Q33222
Qubit™ RNA HS Assay Kit	4–200 ng	100 reactions 500 reactions	Q32852 Q32855
Qubit™ RNA BR Assay Kit	10–1,200 ng	100 reactions 500 reactions	Q10210 Q10211
Qubit™ RNA XR Assay Kit	100–20,000 ng	100 reactions 500 reactions	Q33223 Q33224
Qubit™ microRNA Assay Kit	0.5–150 ng	100 reactions 500 reactions	Q32880 Q32881
Qubit™ 4 System Verification Assay Kit	N/A	50 reactions	Q33237
Qubit™ Flex System Verification Assay Kit	N/A	50 reactions	Q33254

[1] Qubit™ Protein BR Assay Kit is designed for use with Qubit™ 4 only.

Table 3 Instruments

Product	Cat. No.
Qubit™ Flex Fluorometer	Q33327
Qubit™ Flex Fluorometer NGS Starter Kit	Q45893
Qubit™ Flex Fluorometer Quantitation Starter Kit	Q45894
Qubit™ 4 Fluorometer	Q33238
Qubit™ 4 NGS Starter Kit	Q33240
Qubit™ 4 Quantitation Starter Kit	Q33239
Qubit™ 4 RNA IQ Starter Kit	Q33241
Qubit™ 4 Protein BR Starter Kit	A51292

Table 4 Consumables/Accessories

Product	Quantity	Cat. No.
Qubit™ Flex Assay Tube Strips	125 tube strips	Q33252
Qubit™ Assay Tubes	500 tubes	Q32856
Qubit™ 4 Fluorometer International Power Supply (replacement)	1 each	A36204
Qubit™ 4 USB Flash Drive	1 each	Q46009

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision history: Pub. No.MAN0002349

Revision	Date	Description
B.0	February 8, 2022	Updated format and content
A.0	February 2015	New manual

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QUICK REFERENCE

molecular probes® by life technologies™

Qubit® Protein Assay Kit

Cat. nos. Q33211, Q33212 **Pub. no.** MAN0006883 **Revision** 1.0

Detailed protocol is available online at **www.lifetechnologies.com/manuals**, and on the USB drive provided with the Qubit® 2.0 Fluorometer.

WARNING! For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from www.lifetechnologies.com/support.

Chemical	CAS	Warning
Sodium Azide	26628-22-8	Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

1. Set up 3 Assay Tubes for the standards and 1 tube for each user sample.
2. Prepare Qubit® Working Solution by diluting the Qubit® Protein Reagent 1:200 in Qubit® Protein Buffer. Prepare 200 µL of Working Solution for each standard and sample.

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3. Prepare the Assay Tubes (use 0.5-mL PCR tubes) according to the following table.

Volume	Standards	Samples
Working Solution (from step 2)	190 µL	180–199 µL
Standard (from kit)	10 µL	—
User Sample	—	1–20 µL
Total in each Assay Tube	200 µL	200 µL

4. Vortex standards and samples for 2–3 seconds and incubate at room temperature for 15 minutes.

5. Select Protein Assay on the Qubit® 2.0 Fluorometer to calibrate with standards and read the samples.

For support visit lifetechnologies.com/support

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8 February 2013